P43 Consequences of mandatory screening in corneal transplantation

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Background: Human T-cell lymphotropic virus (HTLV) infection is associated with adult T-cell lymphoma (ATL), tropical-spastic-paraparesis (TSP) and HTLV-associated-myelopathy (HAM). In 2004 mandatory screening of corneal donors for HTLV was introduced. We audited HTLV screening of cadaveric corneal donors at the CTS-Manchester Eye Bank.

Patients and Methods: A retrospective analysis of laboratory and transplantation records of corneal donations from August 2004 to December 2005 was carried out. Results of HTLV initial reactivity and subsequent confirmation of test reactivity was compared with Eye Bank data on corneal tissue use.

Results: 1009 corneal donors were tested. 73 (7.2%) were initially reactive. Ethnic origin of all initially reactive samples was Caucasian (100%). 22 (2.1%) were negative on repeat testing. 51 (5%) were referred for confirmation. Of those referred, no samples confirmed positive. 7 (0.7%) samples were insufficient for testing. This equates to a potential donor loss of 160 corneas.

Conclusions: We observed unacceptable wastage of donor tissue on the basis of a screening test with low specificity and poor reproducibility, for a disease of low endemicity in the UK and low risk of subsequent development of disease if transmitted. We question whether mandatory HTLV testing should be suspended until an alternative screening test is available. We suggest that the decision to use corneal tissue should be considered in the light of clinical history and risk factors for HTLV disease.

P44 Greek measles epidemic strain, 2005–2006

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Aim: The purpose of this work was the molecular study of the virus strain that caused the last measles outbreak in Greece.

Methods: Twenty-four saliva specimens were obtained from selected patients serologically confirmed as measles cases between December 2005 and March 2006. Measles virus detection was performed by a nested RT-PCR. The 560 bp segment of the N gene of these MV strains was used for genotyping.

Results: The N gene sequences of the Greek MV strains were identical to each other, so a phylogenetic tree was constructed using one representative MV (ThesGRE/06).

Conclusion: Our data confirmed that the measles virus strain which caused the 2005–2006 outbreak in Greece belonged to genotype D6, had a highly homology with Mvi/Ankara.TUR/29.04 strain and differed by 5.5% from the Edmonston B vaccine strain.

P45 Evaluation of the bioMérieux easyMAG automated extraction system

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Background and Aims: The aim of the evaluation was to compare DNA and RNA quality that had been extracted using the bioMérieux easyMAG system with the automated Roche MagnaPure and manual Qiagen and Gentra methods. To assess the stability of viral RNA extracted using easyMAG.

Methods: The evaluation compared cycle/threshold (CT) or load values of 'real-time' PCR assays (HSV1/2, VZV, CMV, BK, HBV, Enterovirus, Adenovirus, Norovirus genotypes 1/2, Influenza A/B, Neisseria meningitidis) using samples and isolates extracted by easyMAG plus one of Roche MagnaPure, Gentra or Qiagen methods. Samples included EDTA whole blood, plasma, serum, CSF, faeces, NPA, swabs (genital, throat and nose) and urine (n = 181). Stability of Enteroviral RNA was tested by storage at ambient temperature, +4°C, −20°C and −70°C and tested at intervals of zero, two, four and seven days.

Results: Samples extracted by easyMAG, when tested by 'real-time' PCR assays had a comparable level of sensitivity to MagnaPure, Qiagen or Gentra methods. There was no change in the sensitivity of the Enteroviral RT-PCR when tested using RNA extracted by easyMAG and stored at ambient temperature, +4°C, −20°C and −70°C and over the different time intervals.

Discussion and Conclusion: EasyMAG processes different sample types of different input and elution volumes with DNA and RNA extracted concurrently. DNA and RNA quality was comparable with automated and manual extraction methods. A disadvantage of easyMAG is that samples cannot be processed overnight. EasyMAG extracted RNA had excellent stability with no evidence of degradation when stored at ambient temperature for seven days.

P46 Improved laboratory diagnosis of HTLV in corneal transplant donor specimens

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Background: Human T-cell lymphotropic virus (HTLV) infections are reported worldwide, but with specific geographic foci. Maximum seroprevalence ranges from 3–6% in the Caribbean to 30% in rural parts of southern Japan. In the UK the infection is extremely rare. Screening of corneal donors for HTLV has been mandatory since 2004 in the UK, usually using the only CE marked EIA test available in the UK. Testing is normally carried out on cadaveric samples, and a high frequency of repeatedly-reactive results has been observed that have not been confirmed when specimens have been referred to a specialist laboratory.

Aims: In a recent audit of 1009 donors at the CTS-Manchester Eye Bank over a period from August 2004 to December 2005, none of 73 reactive samples were confirmed as HTLV positive. During this time up to 160 corneal transplants were lost as a result of these false positive tests. To determine whether modification of the EIA used for HTLV antibody screening, the use of a gel particle agglutination assay for HTLV antibodies and/or a line immunoassay could significantly reduce the number of reactive samples which need confirmation we conducted a study both using retrospective samples, and prospectively.

Results and Discussion: An improved algorithm using two tests dramatically reduces the number of corneas that have to be discarded thereby improving the service offered to the transplant centre.

P47 The first detection of bovine coronavirus in calves diarrhea in west of Iran

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Background: Bovine coronavirus (BCoV) is associated with severe diarrhea in newborn calves, neonatal calf disease (NCD), winter dysentery (WD) in adult cattle, and respiratory tract infections in calves and feedlot cattle. The BCoV was first recognized as a cause of potentially fatal diarrhea of neonatal calves in 1972. Economically important NCD and WD outbreaks were reported. There is not studying about importance of this virus in calf diarrhea in Iran, Therefore, a study was performed to determine the extent to which BCoV is present in calves with diarrhea from farms in west of Iran.

Method: A total of 108 fecal samples from diarrheic calves were collected and then RNA extracted by QIAamp virus RNA mini kit (Qiagen, UK) as instructed by the manufacture then BCoV RNA was detected by reverse transcription-PCR (RT-PCR) method.

Results: BCoV RNA was detected in 13 of the 108 diarrheic samples (12%) by RT-PCR targeting a 730 bp fragment of the

nucleocapsid (N) gene of BCoV with published primers that could amplify all BCoV strains.

Conclusion and Discussion: This report is the first detection of BCoV in Iran. This study shows that bovine coronavirus is a significant virus in the fecal specimens of calves with diarrhea from farms in west of Iran and thus may be an important pathogen of calves.

P48 Quality control assessment for the PCR diagnosis of TBEV infections

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Background: RT-PCR is an efficient method for an early detection of tick-borne encephalitis virus (TBEV) RNA in blood and serum samples taken prior to the appearance of antibodies. Improving diagnostics is the most important step in detecting and handling this pathogen. Quality control measures are therefore essential tools.

Aims: To assess the diagnostic quality of laboratories we performed an external quality assurance (EQA) programme for the molecular detection of TBE infections.

Methods: A panel of twelve prepared human plasma samples were sent out to be tested for the presence of TBEV-specific RNA. This panel comprised 8 samples spiked with different TBEV strains of the European, Siberian as well as the Far Eastern subtype, including a 10-fold dilution series. Two further samples were prepared as specificity controls containing Louping ill virus and a pool of different other flaviviruses, while two other samples were used as negative controls.

Results: 23 laboratories from 16 European and two non-European countries participated in this EQA. Only two participants have correctly analysed all samples. Nine laboratories are ranging between 91.7% and 75.0% of correct test results, 7 laboratories between 66.7% and 58.3%, and 5 laboratories have less than 50% of correct results with increasing need for improvement of their methodology regarding sensitivity and/or specificity.

Conclusions: The EQA gives a feedback of the quality of the RT-PCR methods used by the participants and indicates a clear need for improvement.

P49 Evaluation of the new NucliSens easyMAG® nucleic acid extraction system

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Aim: The new automated NucliSens easyMAG® system was tested for its ability to extract DNA and RNA from blood plasma, stool, cerebrospinal fluid and throat swab specimens in virus transport medium. The manual Qiagen QIAamp® nucleic extraction systems served as reference systems for comparison.

Methods: For direct comparison, dilution series of virus reference strains or clinical samples were extracted in parallel and amplified in the same run of (RT-)PCR. Clinical materials stored at −80°C were retested by extraction with the NucliSens system and separate amplification.

Results: The NucliSens nucleic acid extraction reagent proved to have an excellent extraction efficacy for DNA and RNA from all the clinical materials tested. The simultaneous extraction of DNA and RNA eliminated the need for separate extraction which is a considerable advantage in routine PCR analysis. Using the NucliSens easyMAG® system no problems with extraction of any of the clinical materials tested were observed. The system handled even “dirty materials” such as stool specimens with a high degree of reproducibility and reliability. No carry-over was observed. No significant discrepancies with respect to the nucleic acid extraction efficacy between the Qiagen and the NucliSens extraction systems were detected for blood plasma, stool and swab specimens in virus transport media. Concentration of nucleic acids by lowering the volume of elution buffer may be indicated for cerebrospinal fluid.

Conclusions: The easyMAG® system proved to be extremely user-friendly. Hands-on time is short; once started the system operates fully automatically which is an important advantage in routine PCR diagnostics.

P50 Diagnosis of Marek’s disease virus in broiler chickens by histopathology and nested-PCR in Iran

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Marek’s disease (MD) continues to be a serious threat to poultry production, despite widespread use of vaccination programmers. Rapid and reliable diagnosis of MD remains an important issue. In this study, Mareks disease virus in broiler chickens is diagnosed by histopathology and molecular methods. A polymerase chain reaction (PCR) and nested-PCR test based on genetic differences between pathogenic and non-pathogenic MDV-1 was utilized. PCR was carried out based on primers from the meq gene. PCR of the DNA extracted from an attenuated strain, amplified a 1200 bp fragment while the DNA from a pathogenic MDV-1 produced a 1062 bp amplicon. In the nested-PCR, the non-pathogenic strain produced a 500 bp DNA fragment and a 300 p band was amplified from the DNA sample from tissue of infected broiler chicken with pathogenic strain. The nested-PCR procedure was found to be a simple and sensitive test for differentiation of pathogenic and non-pathogenic MDV-1 strains and can be used as a rapid diagnostic test.

P51 Simultaneous detection of HSV-1, HSV-2 and VZV in clinical samples by multiplex polymerase chain reaction (MPCR)

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Introduction: Human herpes virus such as Herpes simplex type 1 (HSV-1), type 2 (HSV-2) and Varicella Zoaster (VZV) causing a wild range of acute infections in human which occasionally associated with significant morbidity and mortality. Encephalitis and blindness are the examples of such an occasion.

Aim: The aim of the study was to develop and use a multiplex PCR method for simultaneous detection of HSV-1, HSV-2 and VZV DNAs in different clinical sample. Furthermore, the mPCR results were compared with the results of virus.

Materials and Methods: A total of 93 clinical specimens including 63 skin lesions or vesicles, 28 corneal scraping or conjunctival swabs and 2 CSF samples were collected from patients admitted in Shiraz hospitals. All the specimens were cultured on Vero, HepII and MRC5 cell line. DNA was also purified from specimens by boiling method. Using a specific pair of primer for thymidine kinase gene, both HSV-1 and HSV-2 DNAs were amplified. A set of primer flanking a 208 bp of the DNA-polymerase gene was also used to amplify VZV DNA.

Results: See the table.

Comparison between mPCR results and virus isolation on 93 clinical specimens

Disease	No.	MPCR(+)	MPCR(−)	Culture(+)	Culture(−)
Cutaneous	63	49(77.8)	14(22.2)	32(50.8)	31(49.2)
Ocular	28	17(60.7)	11(39.2)	11(39.2)	17(60.7)
CNS	2	1(50)	1(50)	0(0.00)	2(100)
Total	93	67(72)	26(28)	43(46.2)	50(53.8)

In general the sensitivity of the MPCR for detection of HSV and VZV in clinical samples were 80.9% and 95% respectively. Whereas the sensitivity of cell culture for isolation of HSV and VZV was 62.9%and 72.7% respectively. Interestingly, both HSV and VZV DNAs were detected in 3 out of 93(3.2%) specimens exclusively by MPCR.